BRITISH STANDARD

BS 4172-2:1999

Hand-held pressurized aerosol dispensers against houseflies —

Part 2: Method for determination of insecticidal performance

ICS 55.130; 65.100.10

Committees responsible for this British Standard

The preparation of this British Standard was entrusted to Technical Committee CII/49, Insecticidal aerosols, upon which the following bodies were represented:

British Aerosol Manufacturers Association
British Association for Chemical Specialities
British Pest Control Association
Department of Health — Health Aspects of the Environment and Food Division
Health and Safety Executive
Huntingdon Research Centre
Ministry of Agriculture, Fisheries and Food

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Contents

Foreword

This part of BS 4172 has been prepared by Technical Committee C11/49. It supersedes BS 4172-2:1993 which is withdrawn.

The principal changes in this edition are as follows.

a) The formulation used in the reference dispensers has been revised to eliminate chlorofluorocarbons (CFCs).

b) Appropriate changes have been made in the formulation and preparation of the reference dispensers and in the specification of the reference dispenser container valves.

c) Terminology has been revised to improve conformity with that used in related agricultural and horticultural applications. For example, the noun “spray” is replaced by “aerosol” and “efficiency” is replaced with “performance”.

d) The cone angle test has been deleted.

Annex A and annex E are informative. Annex B, annex C and annex D are normative.

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Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, pages 1 to 14, an inside back cover and a back cover.

1 Scope

This part of BS 4172 describes a method for determining the insecticidal performance of hand-held pressurized aerosol dispensers (hereafter referred to as "dispensers") for indoor use as space treatments against houseflies.

The method does not apply to metered-valve or total-release dispensers.

NOTE The principle of the method may be used for the determination of insecticidal performance of space treatments against other flying insects. However, the method may not be valid for products intended for use against strains tolerant of pyrethroid insecticides.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of this British Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. For undated references, the latest edition of the publication applies.

BS 4172-1:1998, *Hand-held pressurized dispensers against houseflies — Specification for insecticidal performance.*

3 Definitions

3.1

block

group of dispensers for testing

3.2

candidate

dispenser under test

3.3

dispenser

container incorporating an aerosol valve and holding a product at a pressure greater than atmospheric pressure, from which the product is discharged by means of a propellant when the valve is opened

NOTE Dispensers are intended for disposal when exhausted and not for refilling.

3.4

knockdown

measure of the percentage by number of dead and moribund flies or those unable to fly or walk in a co-ordinated fashion

NOTE Assessment can be made either by counting at a given time after spray application, or by assessing the percentage in the population.

3.5

study

tests required to compare the performance of the candidate(s) with that of the reference dispenser

3.6

KT_{50}

time, in minutes, taken for the knockdown of 50 % of the test insects

3.7

24 h knockdown

percentage by number of test insects knocked down 24 h after the start of the test

4 Principle

A dispenser is discharged into a test chamber containing an internationally recognized susceptible strain of the housefly *Musca domestica* L. under controlled conditions. Knockdown is assessed at:

— up to 15 min; and

— at 24 h after discharge.

The results of replicate determinations of knockdown (**7.3**) for the candidate and calibrated reference dispensers are compared statistically (see clause **8**).

5 Materials and apparatus

5.1 *Test insects*, of adult *Musca domestica* L. of an internationally recognized susceptible strain, of mixed sex and 3 days to 6 days old at the time of test.

NOTE 1 A recommended breeding regime is given in annex A.

The average mass of at least three random samples, each sample containing a total of 100 clean and dry pupae, 1 day to 2 days old and destined for use in the test, shall be between 1.75 g and 2.5 g.

At the beginning of the study, the vigour and susceptibility of the test insects shall be checked using a reference dispenser prepared in accordance with annex B.

When determined in accordance with clause **7**, the reference dispenser shall have a KT_{50} of 6.5 min $\pm$ 2 min and 24 h knockdown between 50 % and 99.0 % at a dosage rate of 5.3 g $\pm$ 0.5 g of aerosol formulation per 50.0 m^3.

If an anaesthetic is used to handle the test insects, a 12 h recovery period prior to conditioning shall be allowed.

NOTE 2 It is recommended that carbon dioxide is used as the anaesthetic.

The test insects shall be conditioned for at least 3 h before the test at a light intensity approximating to that of the test chamber (**5.3**), at a temperature of 26 °C $\pm$ 2 °C and at a relative humidity of 45 % to 75 %.

NOTE 3 The usual food should be provided.

5.2 *Six or more reference dispensers*, prepared in accordance with annex B and conditioned at 26 °C $\pm$ 2 °C for at least 30 min before use.

5.3 *Test chamber*, of a volume not less than 25 m^3 and not greater than 60 m^3. The ratio of the length to the width shall be not greater than 1.2:1.0. The chamber shall be 2.4 m to 2.8 m in height.

The floor shall be covered with non-glazed paper which shall be replaced for the start of each day (see **7.1**). Other surfaces shall be of a cleanable material or covered by a replaceable lining such as cling film wrap. Illumination shall be by fluorescent lighting sited to eliminate shadows.

The chamber shall be maintained at a temperature of 26 °C ± 2 °C and at a relative humidity of 45 % to 75 % during the period of testing. Temperature gradients shall be avoided and any air-conditioning apparatus present shall be turned off. If mobile equipment is used, it shall be removed before commencing the test. The chamber shall be designed not to give rise to convection currents during the test. The extract vent shall be covered during the test period to prevent flies gaining access. The chamber shall be ventilated after each test to ensure a minimum of four complete air changes before the start of a subsequent test.

5.4 *Storage containers*, typically 0.5 l to 1 l, with clear lids, having the same size and construction for each study.

6 Sampling and treatment of the candidate

6.1 Sampling

Select at least six dispensers at random from those available for the test.

6.2 Treatment

Ensure that each candidate contains a mass of 50 % to 60 % of its declared contents, the estimation for which shall be made by weighing against a previously weighed empty can and valve assembly of similar size. Attain this level of fill by discharging the dispenser repeatedly for periods individually not exceeding 10 s. Shake the dispenser to restore equilibrium before each discharge.

Condition the candidate for at least 30 min at 26 °C ± 2 °C then calibrate the candidate for its discharge rate by weighing before and after discharge over a measured time interval of approximately 5 s.

7 Procedure

WARNING. Operators discharging dispensers in test chambers should be equipped with fully effective personal protective equipment.

7.1 Test chamber contamination checks

Check the test chamber for insecticidal contamination before the commencement of the study and at the end of every day.

Release at least 100 test insects (**5.1**) of mixed sex into the chamber and leave them there for 15 min with the ventilation turned off and the extract vent covered. Collect and hold the test insects in accordance with **7.3**.

If there is more than 5 % knockdown within 15 min, or more than 10 % after 18 h ± 6 h, clean the chamber and replace the floor paper (see **5.3**). Repeat the contamination test procedure and invalidate the results of all tests carried out during the previous 24 h period.

7.2 Conduct of study

Perform one valid determination (see **7.1**) for at least six of the reference dispensers, (**5.2**) and the same number of candidates (see clause **6**) in the study. Use a randomized block design to determine the order of testing within each day.

NOTE A block design ensures that the same number of candidates and reference dispensers are tested on each day. It also ensures that the ordering *CCCRRR*, which has potential for bias, never occurs, where *R* is a reference dispenser and *C* is a candidate dispenser.

Two possible randomizations are given below for the case of six determinations per day:

CRRCRC (with one candidate);

$C_1RC_2\ RC_1C_2$ (with two candidates).

7.3 Determination

Weigh the reference dispenser (**5.2**) and the candidate (**6.2**) to the nearest 0.01 g.

Either discharge the reference dispenser at a rate of 5.3 g ± 0.5 g per 50.0 m^3 or discharge the candidate at the dosage recommended on the product label, or by the manufacturer.

Hold the dispenser upright and, walking backwards, continuously discharge it down the longer axis of the test chamber (**5.3**), moving it from side to side at a height of approximately 1.8 m, for the time necessary to give the required dosage as described in the paragraph above. Do not discharge the dispenser at a distance of less than 1 m from any vertical surface.

Release a minimum of 100 mixed sex flies (**5.1**) at floor level in the centre of the chamber over a maximum period of 10 s, beginning at 20 s after commencement of discharge of the dispenser. Take at least five counts of knockdown, at regular intervals during a period of 10 min from the time of release of the flies. Should there be less than 70 % knockdown at 10 min, make a further two counts at 12 min and 15 min. Ventilate the chamber after completing the final count.

At the end of the exposure period, collect all the flies, both knocked down and flying, by gentle suction or other means, into clean containers (**5.4**). Avoid overcrowding of flies. Re-weigh the dispenser to the nearest 0.01 g.

If the required dosage rate is not achieved, invalidate the test result.

After collection, insert a separate feed container holding approximately 10 ml of 5 % (*m/m*) sugar solution. Hold the storage container for 24 h ± 6 h in a room maintained at a temperature of 26 °C ± 2 °C and a relative humidity of 45 % to 75 %. Assess the 24 h knockdown, and express as a percentage of the population.

While assessing the 24 h knockdown, check the sex ratio and if this falls outside the range of 1.2:1.0 to 1.0:0.83, invalidate the test result.

8 Calculation and expression of results

8.1 General

A statistical treatment of the results from the study is described in **8.2** and **8.3**. It is sufficiently accurate except in those cases where it is found that greater correlation exists within days than between days. In this case, conduct a within-block analysis to obtain the values for the confidence limits U_{95} and L_{95} given in **8.2.7** and **8.3.7** directly.

NOTE It is strongly recommended that all testing of candidates and reference dispensers is carried out within six days to reduce the requirement for within-block analysis.

8.2 KT_{50} results

8.2.1 Calculate the percentage values for knockdown. Plot the percentage knockdown values against time for each dispenser.

8.2.2 Determine the KT_{50} by linear interpolation of the two points either side of, but not on, the 50 % ordinate of the graph. Transform each value of KT_{50} to $\log_{10}$ scale.

NOTE Regression techniques are inappropriate because of the non-independence of results at individual time points.

8.2.3 On the $\log_{10}$ scale, calculate the mean $\log_{10} KT_{50}$ for the reference and candidate dispensers, $\overline{x}_r$ and $\overline{x}_c$ and calculate the standard error, se($\overline{x}$) for each mean value from the following general equation:

$$\text{se}(\overline{x}) = \frac{s}{\sqrt{n}}$$

where

s is the standard deviation of the $\log_{10} KT_{50}$;

n is the number of dispensers.

8.2.4 Calculate the difference, δ, in mean $\log_{10} KT_{50}$ between candidate and reference dispensers, from the equation:

$$\delta = \overline{x}_c - \overline{x}_r$$

8.2.5 Divide the larger value of $\{\text{se}(\overline{x})\}^2$ by the smaller. If the result is less than, or equal to the appropriate critical value given in Table 1, continue the analysis as described in **8.2.6** and **8.2.7**. If the result exceeds the appropriate critical value, estimate the confidence limits by an alternative method, stating the method used in the test report (see clause **9**).

Table 1 — Critical values for the 95 % two-tailed *F*-distribution

Number of dispensers, n, for the reference or candidate	Critical value
6	7.15
7	5.82
8	4.99
9	4.43
10	4.03
11	3.72
12	3.48
13	3.28
14	3.12
15	2.98
16	2.86
17	2.77
18	2.68
19	2.60
20	2.53

8.2.6 Calculate the standard error of the difference, se(δ), from the following general equation (with variables defined in **8.2.4**):

$$\text{se}(\delta) = [\{\text{se}(\overline{x}_r)\}^2 + \{\text{se}(\overline{x}_c)\}^2]^{½}$$

8.2.7 Calculate the lower and upper 95 % confidence limit values, L_{95} and U_{95}, for δ, from the following general equations:

$$L_{95} = \delta - \{t \times \text{se}(\delta)\}$$

$$U_{95} = \delta + \{t \times \text{se}(\delta)\}$$

where

t is the Student's multiplier, chosen from Table 2.

NOTE The L_{95} value is used to decide whether or not to increase the number of dispensers in the study (see **8.4.2**).

Table 2 — Values for the 95 % two-tailed *t*-distribution

Number of dispensers, n, for the reference or candidate	Student's multiplier, t
6	2.228
7	2.179
8	2.145
9	2.120
10	2.101
11	2.086
12	2.074
13	2.064
14	2.056
15	2.048
16	2.042
17	2.037
18	2.032
19	2.028
20	2.025

8.2.8 Calculate the antilog$_{10}$ of the following values, giving results in minutes and as percentages:

a) $\overline{x}_r$ and $\overline{x}_c$, to give the mean KT_{50} value, expressed in minutes, for reference and candidate dispensers;

b) δ, to give the ratio (fold difference) of the candidate mean KT_{50} value relative to the reference mean value;

c) L_{95} and U_{95}, to give the lower and upper 95 % confidence limits for the ratio value obtained in item b).

8.3 24 h knockdown results

8.3.1 Record the percentage values, Y, for knockdown at 24 h ± 6 h (see **7.3**).

8.3.2 Determine the arcsin values, x in degrees, for each value (**8.3.1**) from the following equation:

$$x = \arcsin\sqrt{\left(\frac{Y}{100}\right)}$$

If percentage knockdown is 100 %, then calculate the arcsin value using the following general equation:

$$x = \arcsin\sqrt{\left(\frac{n_f - 0.25}{n_f}\right)}$$

where

n_f is the number of flies used in the determination.

8.3.3 On the arcsin scale, calculate the mean results, $\overline{x}_r$ and $\overline{x}_c$, then calculate the standard error for each mean value, se($\overline{x}_r$) and se($\overline{x}_c$), from the general equation given in **8.2.3**.

8.3.4 Calculate the difference, δ as follows:

$$\delta = \overline{x}_r - \overline{x}_c$$

NOTE The order of subtraction in this case is the reverse of that given in **8.2.4**. A negative difference means the candidate has a larger percentage 24 h knockdown value than the reference dispenser.

8.3.5 Divide the larger value of $\{se(\overline{x}_r)\}^2$ and $\{se(\overline{x}_c)\}^2$, by the smaller. If the result is less than, or equal to, the appropriate critical value given in Table 1, continue the analysis as described in **8.3.6** and **8.3.7**. If the result exceeds the appropriate critical value, estimate the confidence limits by an alternative method, stating the method used in the test report (see clause **9**).

8.3.6 Calculate the standard error of the difference, se(δ), from the general equation given in **8.2.6**.

8.3.7 Calculate the lower and upper 95 % confidence limit values, L_{95} and U_{95}, for δ from the general equations given in **8.2.7**.

8.3.8 Backtransform, $\overline{x}_r$ and $\overline{x}_c$, to give the mean percentage 24 h knockdown for the reference and candidate dispensers, using the following equation:

$$\overline{Y} = \{\sin(\overline{x})\}^2 \times 100$$

Express the difference in percentage 24 h knockdown between reference and candidate dispensers, by subtracting $\overline{Y}_c$ from $\overline{Y}_r$, with both values obtained as above.

NOTE See annex E to aid interpretation.

8.4 Assessment of insecticidal performance

8.4.1 If neither of the upper 95 % confidence limit values, U_{95}, for the difference between candidate and reference dispensers obtained in **8.2.7** and **8.3.7**, is greater than the target values of the conformity criteria, A and B respectively, then the insecticidal performance of the candidate shall be rated as "not worse" than that of the reference dispenser (see **E.4**).

NOTE Figure E.1 shows an example where U_{95} is less than both A and B.

8.4.2 Additional dispensers may be tested if the values for L_{95} and U_{95} fall on each side of A and B. In this case reassess the total results of the study in accordance with clause **8**.

NOTE 1 Figure E.3 shows an example where L_{95} and U_{95} lie to either side of A and B.

NOTE 2 A consequence of this method of assessment following the statistical treatment is that a candidate with a wide range of results with mean knockdown values even slightly better than those for the reference dispenser may still fail to conform with the criteria chosen.

9 Test report

The test report shall include the following information:

a) a complete identification of the candidate;

b) a reference to this British Standard, i.e. BS 4172-2:1999, with a statement detailing any variation(s) from the procedure;

c) the date and duration of the study;

d) the strain of susceptible houseflies, i.e. the source of the culture;

e) the original and the usage mass ranges of the reference dispensers and the candidates;

f) number, n, of reference and candidate dispensers;

g) the mean results, $\overline{x}$, for the KT_{50} and the 24 h knockdown values on their respective transformed scales, obtained in accordance with **8.2.3** and **8.3.3** respectively for both the reference dispenser and the candidate;

h) the difference, δ, between the reference dispenser and candidate for both KT_{50} and percentage 24 h knockdown on their respective transformed scales in accordance with **8.2.4** and **8.3.4** respectively;

i) the values of L_{95} and U_{95} obtained in accordance with **8.2.7**, or estimated (see **8.2.5**) i.e. the 95 % confidence interval of the difference between the mean KT_{50} values of the reference dispenser and candidate expressed on the $\log_{10}$ scale;

j) the values of L_{95} and U_{95} obtained in accordance with **8.3.7**, or estimated (see **8.3.5**), i.e. the 95 % confidence interval of the differences between the mean percentage 24 h knockdown values of the reference dispenser and candidate expressed on the arcsin scale;

k) the mean results for KT_{50} in minutes and the 24 h knockdown values as percentages as obtained in **8.2.8** and **8.3.8** respectively for both the reference dispenser and the candidate;

l) the results in accordance with **8.4** as “worse” or “not worse” than the reference dispenser.

Annex A (informative)
Recommended method for breeding houseflies

A.1 Principle

Successive generations of *Musca domestica* L. are separated and grown as new cultures over a 14 day cycle under controlled insectary conditions.

A.2 Materials

A.2.1 *General*. Use animal feed grade materials unless otherwise stated.

A.2.2 *Cows' milk*, pasteurized.

A.2.3 *Formaldehyde solution*, reagent grade, c(HCHO) = 400 g/l.

A.2.4 *Bakers' yeast suspension*, disperse 230 g of fresh, live bakers' yeast in 1 l of water and store for a maximum of 7 days in a refrigerator at 5 °C ± 3 °C.

A.2.5 *Soya flour*, containing 20 % oil by mass, heat treated.

A.2.6 *Malt extract*, with cod liver oil B.P.

A.2.7 *Larval medium*, prepared freshly by thoroughly mixing the ingredients given in Table A.1 in the order and proportions indicated.

Table A.1 — Fresh larval medium

Ingredient	Proportion by mass fraction %
Wheat bran	24.0
Grass meal	6.0
Malt extract (**A.2.6**)	3.0
Soya flour (**A.2.5**)	2.0
Yeast suspension (**A.2.4**)	0.8
Water	64.2
Total	100.0

A.3 Apparatus

A.3.1 *Insectary*, maintained at a temperature of 26 °C ± 2 °C and at a relative humidity of 45 % to 75 %. Illuminate the insectary with fluorescent tubes for 12 h per day for maximum egg production.

A.3.2 *Insectary cages*, covered with wire or plastic gauze 0.06 mm in diameter [23 standard wire gauge (SWG)] and 2.5 mm aperture.

A.4 Procedure

A.4.1 House the parent flies (**5.1**) in the cages (**A.3.2**). Destroy the parent flies and start new cultures every 14 days with approximately 1 500 pupae per 0.03 m^3 of cage. Provide the flies with solid sugar and a mixture of equal parts of the cows' milk (**A.2.2**) and water containing 0.05 % by volume of the formaldehyde solution (**A.2.3**), as a preservative. Add a shallow container of the larval medium (**A.2.7**) daily for oviposition.

A.4.2 Separate the eggs from the medium by flotation with water. Using a pipette, add them to the culture medium at the rate of approximately 0.1 ml (or 400 eggs) per 300 g of fresh medium, stored in containers covered with fine muslin.

A.4.3 Remove the pupae from the surface layers 8 days after seeding, spread thinly on trays and dry in a current of air for up to 4 h.

A.4.4 Separate the pupae from the medium by gentle winnowing and place approximately 1 000 pupae per 0.03 m^3 into bags or cages. Supply with food.

NOTE Food should be replaced daily. A tube of approximately 40 ml containing cotton wool soaked in a mixture of equal parts of milk and water is sufficient to feed approximately 300 flies for 24 h.

Annex B (normative)
Method for preparation of the reference dispensers

B.1 Principle

Dispensers are prepared by pressure-filling propellant through a specified valve which has been crimped onto a specified container following the addition of filling solution containing the active components.

The dispensers are selected in accordance with the requirements (see **B.5**) for composition, droplet size and discharge rate.

B.2 Reagents

B.2.1 *General*

During the preparation, use only reagents of technical grade or higher purity.

B.2.2 *S-bioallethrin*, 95 % (*m/m*) minimum.

NOTE The chemical name for *S-bioallethrin* is (S)-3-allyl-2-methyl-4-oxocyclopent-2-enyl(1*R*,3*R*)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate.

B.2.3 *Bioresmethrin*, 93 % (*m/m*) minimum.

NOTE The chemical name for bioresmethrin is 5-benzyl-3-furylmethyl(1*R*,3*R*)-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropanecarboxylate.

B.2.4 *Deodorized kerosene*

NOTE Deodorized kerosene is a mixture of aliphatic hydrocarbons derived from the dearomatization of gasoline and has a typical boiling range of 190 °C to 250 °C.

B.2.5 *Propellant (butane/propane mixture)*, comprising a blend of propane, iso-butane and *n*-butane, having a vapour pressure at 25 °C of 330 kPa ± 20 kPa.

B.3 Materials and apparatus

B.3.1 *General ordinary laboratory apparatus*, amber glass storage vessels and the following.

B.3.2 *Aerosol container (can)*, made of three piece tinplate, internally plain.

B.3.3 *Valve*, having the following characteristics and nominal dimensions:

type:	male;
stem orifice:	1 × 0.51 mm diameter;
housing:	2.03 mm;
dip tube:	standard (i.e. not capillary), length appropriate to can size;
actuator:	single piece; orifice diameter 0.41 mm, straight taper.

B.4 Procedure

B.4.1 *General*

Carry out all necessary weighings to within ±0.1 % relative except for propellant filling which may be ±1 % relative.

B.4.2 *Preparation of filling solution*

Thoroughly mix the ingredients listed in Table B.1 in the order and proportions indicated so that a uniform solution of the required amount is obtained.

Table B.1 — Composition of filling solution

Component	Mass fraction %
S-bioallethrin (**B.2.2**) (as pure)	0.286
Bioresmethrin (**B.2.3**) (as pure)	0.071
Deodorized kerosene (**B.2.4**)	to 100.000

Determine the *S*-bioallethrin and bioresmethrin content of the filling solution as described in annex C.

NOTE A certificate of analysis may be provided with the filled dispensers.

B.4.3 *Preparation of aerosol formulation*

Prepare the required amount of aerosol formulation in accordance with the proportions indicated in Table B.2.

Table B.2 — Composition of aerosol formulation

Component	Mass fraction %
Filling solution (see **B.4.2**)	35.0
Butane/propane mixture (**B.2.5**)	65.0
Total	100.0

Weigh the filling solution into the aerosol container (**B.3.2**). Carefully displace air from the headspace by discharging approximately 2 g liquid propellant (**B.2.5**) from a separate dispenser containing only propellant. Allow approximately 2 s for the propellant to evaporate and then crimp the aerosol valve onto the container.

Pressure-fill the appropriate quantity of propellant (**B.2.5**) through the aerosol valve.

NOTE Attention is drawn to the *Prescribed Quantities Directive* 80/232/EEC [1] for the particular gross capacity of the container used. The filling weight, (in grams), should be taken as 0.625*V*, where *V* is the product volume in millilitres. For convenience the net weight and net volume should be marked on the dispenser to help determine when the contents fall below 20 % of their initial volume, at which point the dispenser should be discarded (see **B.5.1**).

B.4.4 *Storage of the reference dispenser*

Store each reference dispenser, before and between tests, out of direct sunlight and at 20 °C ± 5 °C.

B.5 Checking each reference dispenser

B.5.1 *General*

Ensure that each reference dispenser is at least 20 % full by estimating the net mass against a previously weighed empty can and valve assembly of similar size. Ensure that any dispenser not conforming to the requirements given in **B.5.2** to **B.5.5** is discarded.

If a dispenser has not previously been discharged, then before carrying out any measurements discharge the dispenser for approximately 1 s to expel any non-representative material from the dip tube (e.g. propellant from the pressure-filling).

B.5.2 *Droplet size distribution*

Determine the droplet size distribution of the aerosol from the dispenser in accordance with annex D.

The aerosol shall have a volume median diameter D_{med} of not less than 27 μm and not greater than 35 μm.

NOTE Propellant and solvent vapours frequently cause an overestimation of the large droplets in the top size range detected by the laser diffraction instrument. It is then normal practice to eliminate this effect with the command *Kill (1,0)* whereupon the error of fit to the Rosin Rammler distribution is significantly reduced; this changes the value obtained for the volume median diameter.

B.5.3 ***Discharge rate***

Shake the dispenser thoroughly to restore equilibrium before discharge. Determine the discharge rate by weighing the dispenser, to the nearest 0.01 g, before and after discharging for a period of 5 s at a temperature of 25 °C ± 2 °C.

The discharge rate shall be 1.1 g/s ± 0.2 g/s.

B.5.4 ***Content of active ingredients***

Determine the content of *S*-bioallethrin and bioresmethrin in accordance with annex C.

The minimum contents of active ingredients shall be as follows:

S-bioallethrin: 0.10 ± 0.01 % (*m/m*) (as pure);

bioresmethrin: 0.025 % ± 0.004 % (*m/m*) (as pure).

Annex C (normative)

Method for determination of *S*-bioallethrin and bioresmethrin content of the reference dispensers

C.1 Principle

The *S*-bioallethrin and bioresmethrin content is determined using a gas chromatograph fitted with a flame-ionization detector.

NOTE The *S*-bioallethrin and bioresmethrin content of the filling solution should be determined and the filling solution approved before the reference dispensers are prepared. The *S*-bioallethrin and bioresmethrin content of the filled dispensers may be determined as a final check.

C.2 Reagents

C.2.1 *Acetone*, of recognized analytical reagent grade.

C.2.2 *S-bioallethrin*, reference material of known purity (see **B.2.2**).

C.2.3 *Bioresmethrin*, reference material of known purity (see **B.2.3**), stored in amber glassware.

NOTE Bioresmethrin is photochemically unstable and will degrade rapidly in direct light.

C.3 Apparatus

C.3.1 *Ordinary laboratory apparatus.*

C.3.2 *Gas chromatograph*, fitted with a flame-ionization detector, linked to an electronic integrator and recorder and set up as described in **C.3.3**.

C.3.3 *Column and column conditions*

C.3.3.1 *Single packed glass column*, of 1 m to 2 m in length and 2 mm to 4 mm internal diameter.

Condition the disconnected, freshly packed column immediately prior to the determination by heating to 300 °C for 1 h whilst purging with nitrogen gas (see **C.3.3.4**) at a rate of 20 ml/min to 60 ml/min.

C.3.3.2 *Column packing*, typically 2 % (*m/m*), of dimethyl silicone fluid as the stationary phase on an acid-washed, silanized diatomaceous earth support.

NOTE 1 The proportion of the stationary phase will depend on the density of the support used.

NOTE 2 Stationary phases found to be suitable include OV-101, SE-30 and OV-1.

NOTE 3 A support found to be suitable is Gas Chrom Q, 80 to 100 mesh.

C.3.3.3 *Temperatures*, typically as follows:

a)	column:	210 °C;
b)	injection point:	230 °C;
c)	detector:	250 °C.

C.3.3.4 *Gases and flow rates*, as follows:

a)	carrier gas:	nitrogen (thoroughly dried and with an oxygen content of less than 10 mg/kg) at approximately 60 ml/min;
b)	fuel gases:	hydrogen and air (free from organic impurities) at optimum flow rates recommended by the instrument manufacturer.

C.3.3.5 *Glass injection syringe*, of 10 µl capacity.

C.3.4 *Two stoppered one-mark volumetric flasks*, of 25 ml capacity, made from amber glass.

C.3.5 *Two one-mark volumetric flasks*, of 100 ml capacity, made from amber glass.

C.3.6 *Two round-bottomed flasks*, of 250 ml capacity, made from amber glass (not required for filling solution analysis)

C.3.7 *Rotary evaporator* (not required for filling solution analysis).

C.4 Preparation of solutions

C.4.1 ***Calibration solution***

Weigh, (to the nearest 0.000 1 g), approximately 0.1 g of the *S*-bioallethrin (**C.2.2**) and 0.025 g of the bioresmethrin (**C.2.3**) and place in a 100 ml one-mark volumetric flask (**C.3.5**). Dilute to the mark with acetone (**C.2.1**) and mix thoroughly.

C.4.2 ***Test solution***

C.4.2.1 *Filling solution test sample*

Weigh, (to the nearest 0.01 g), approximately 8.75 g filling solution (**B.4.2**) and place in a 25 ml flask (**C.3.4**). Dilute to the mark with acetone and mix thoroughly.

C.4.2.2 *Reference dispenser test sample*

Carry out the following procedure in a fume-cupboard or safety cabinet.

Mix the contents of the dispenser thoroughly by making several inversions, then discharge the dispenser in an upright position for approximately 1 s to dispel any non-representative product from the dip-tube. Remove the actuator, attach an actuator fitted with a capillary tube at least 100 mm in length and weigh to the nearest 0.01 g.

Insert the capillary tube into a 250 ml round-bottomed flask until it touches the bottom. Using the dispenser in a near upright position, depress the actuator gently several times until approximately 25 g of the product has been discharged, swirling the flask gently between each depression to remove propellant. Remove the dip tube and wash it through with acetone (**C.2.1**), adding the washings to the flask. Dry the capillary tube with a current of air.

Re-weigh the dispenser and capillary tube to the nearest 0.01 g and record the mass of the transferred product.

Place the flask in an ultrasonic bath, or shake gently in a water bath, at approximately 40 °C to assist propellant removal. Once the evolution has slowed, attach the flask to the rotary evaporator (**C.3.7**) and remove the remaining propellant under vacuum at approximately 50 °C, taking care to avoid boiling over.

Quantitatively transfer the contents of the round-bottomed flask to a 25 ml volumetric flask (**C.3.4**) by means of a glass funnel and Pasteur pipette. Rinse the round-bottomed flask with 2 ml to 3 ml acetone and add the washings to the volumetric flask. Dilute to the mark with acetone and mix thoroughly.

C.5 Procedure

C.5.1 Inject 5 µl of the calibration solution (**C.4.1**) on to the column (**C.3.3**) and record the chromatogram, selecting attenuation settings to optimize the integrals for both components.

NOTE Approximate retention times, subject to the particular conditions chosen (see **C.3.3**), are as follows:

S-bioallethrin: 2.75 min;
bioresmethrin: 6.0 min.

Repeat this procedure and calculate the arithmetic mean integral values for each component.

C.5.2 Inject 5 µl of the test solution (**C.4.2.1** or **C.4.2.2**) on to the column and record the chromatogram and associated integral counts under the same conditions as described in **C.5.1**.

Repeat this procedure and calculate the arithmetic mean integral values for each component.

C.6 Calculation

Calculate the percentage by mass of *S*-bioallethrin and bioresmethrin, m_x, in the filling solution or reference dispenser from the following general equation:

$$m_x = \frac{0.25TWP}{SM}$$

where

T is the mean integral count for the area of the *S*-bioallethrin or bioresmethrin peak for the test solution (**C.5.2**);

W is the mass in grams of *S*-bioallethrin or bioresmethrin in the calibration solution (see **C.4.1**);

P is the purity in percentage by mass of the *S*-bioallethrin (**C.2.2**) or bioresmethrin (**C.2.3**);

S is the mean integral count for the area of the *S*-bioallethrin or bioresmethrin peak for the calibration solution (**C.5.1**);

M is the mass in grams of the sample in the test solution.

C.7 Test report

The test report shall include the following information:

a) the result and the date of the test;

b) the individual integral counts obtained in accordance with **C.5**;

c) any operation not described in this method;

d) the identification of the reference dispenser.

Annex D (normative)

Method for determination of droplet size of the aerosol from the reference dispenser

D.1 Principle

The reference dispensers are preconditioned to a specified temperature. The droplet size of the aerosol is determined by an analyser, using monochromatic light diffraction.

D.2 Apparatus

D.2.1 *Laboratory water bath*, of sufficient size to hold the dispenser and capable of controlling the temperature at 25 °C ± 1 °C.

D.2.2 *Laboratory scissor jack*, to support the dispenser.

D.2.3 *Droplet size analyser*[1)], operating in the droplet size range 5 μm to 500 μm, capable of illuminating an aerosol plume with a laser beam of wavelength 632.8 nm, measuring the diffraction pattern and, by use of the Rosin Rammler model for droplet size distribution, computing the required parameters.

NOTE Guidance for safe use of laser systems is given in BS EN 60825.

D.3 Conditioning the reference dispenser

Immerse the dispenser, prepared in accordance with **B.4.2**, and filled to at least 20 % (*V/V*), for at least 30 min in the water bath (**D.2.1**) set at 25 °C ± 1 °C prior to taking each measurement.

Recondition the dispenser for at least 5 min in the water bath between each measurement.

D.4 Procedure

D.4.1 *Determination*

Adjust the droplet size analyser (**D.2.3**) in accordance with the manufacturer's instructions, ensuring that the baseline obtained in the absence of a sample is within the manufacturer's recommendations.

Thoroughly shake the conditioned dispenser and discharge for approximately 1 s to expel non-representative material from the dip tube.

Secure the dispenser, conditioned in accordance with **D.3**, with the laboratory scissor jack (**D.2.2**). Actuate the dispenser, directing the discharge cone so that its axis intersects the laser beam perpendicularly at a distance of 400 mm from the aerosol actuator button and a distance not greater than the focal length of the lens from the front element of the lens.

NOTE A lens hood may be used to prevent the aerosol hitting the front element of the lens.

Establish a stable emission, then record the diffraction pattern for approximately 3 s.

Compute the Rosin Rammler parameters, X and N, for the droplet size distribution of the aerosol discharge from the dispenser in accordance with the manufacturer's instructions.

D.4.2 *Number of determinations*

Carry out two determinations, each giving values for droplet size distribution with an acceptable error as defined by the manufacturer's instructions. Repeat any measurement which fails to give such an acceptable error.

EXAMPLE

The values of the parameters, X and N, computed by the analyser, together with the log error, E, between the actual and computed diffraction patterns are recorded. If the log error, E, is greater than 5.5 then this signifies a poor fit and the measurement has to be repeated.

D.5 Calculation and expression of results

For each of the duplicate determinations, calculate the volume median diameter, D_{med}, in micrometres, from the following general equation:

$$D_{med} = X\,(\log_e 2)^{\frac{1}{N}}$$

Express the final result as the arithmetic mean value of D_{med} of the two determinations.

D.6 Test report

The test report shall include the following information:

a) the result and date of the test;

b) the duplicate values of D_{med}, X, N;

c) any operation not described in this method;

d) the identification of the reference dispenser.

Annex E (informative)
Example of a statistical treatment of results

E.1 General

One of the assumptions behind the treatment of data described in this part of BS 4172 is that of equal variances between the treated groups. Experience suggests that this is not the case for data for either KT_{50} or percentage 24 h knockdown. However, transforming the KT_{50} data onto the $\log_{10}$ scale and converting percentage 24 h knockdown onto the arcsin scale does attempt to stabilize them. In addition, analysis using these scales attempts to take account of differences in response level between test laboratories. This latter point should ensure that the decision whether or not a particular candidate conforms to the chosen criteria is independent of the laboratory in which the test was carried out.

An example of the calculations to be carried out, on a step by step basis, is given in **E.2** and **E.3**, followed by an interpretation in **E.4**.

NOTE The unit "min" (for minutes) has been omitted from the calculations and text of annex E for clarity.

E.2 KT_{50} results

E.2.1 An example of the calculation of KT_{50} results, to be carried out on a step by step basis, is given in **E.2.1** to **E.2.7**, using the values in Tables E.1 and E.2.

[1)] The Malvern ST series of droplet size analysers are examples of suitable products available commercially. This information is given for the convenience of users and does not constitute an endorsement by BSI of these products.

Table E.1 — Example of KT_{50} results

Time in minutes		Log_{10} scale	
Reference dispenser	Candidate	Reference dispenser	Candidate
6.0	7.2	0.778	0.857
6.5	8.3	0.813	0.919
5.5	9.6	0.740	0.982
7.0	5.6	0.845	0.748
6.0	9.1	0.778	0.959
5.0	5.1	0.699	0.708

E.2.2 Calculate the mean $\log_{10}$ KT_{50}, $\bar{x}$, and its standard error, se($\bar{x}$), (see **8.2.3**) as shown in Table E.2.

Table E.2 — Mean $\log_{10}$ values and standard errors

	Reference dispenser	Candidate
$\bar{x}$	0.776	0.862
se($\bar{x}$)	0.021	0.046

E.2.3 Calculate the difference, δ, between the candidate and the reference dispenser (see **8.2.4**).

$$\delta = 0.862 - 0.776$$
$$= 0.086$$

E.2.4 Check for homogeneity of variances (see **8.2.5**).

$$\frac{0.46^2}{0.021^2} = 4.80$$

4.80 is less than 7.15 therefore continue the analysis.

E.2.5 Calculate the standard error of the difference, se(δ), (see **8.2.6**).

$$\text{se}(\delta) = \sqrt{(0.046^2 + 0.021^2)}$$
$$= 0.051$$

E.2.6 Calculate the lower and upper 95 % confidence limits of the difference, L_{95} and U_{95}, (see **8.2.7**).

$$L_{95} = 0.086 - (2.228 \times 0.051)$$
$$= -0.028$$
$$U_{95} = 0.086 - (2.228 \times 0.051)$$
$$= -0.200$$

E.2.7 Calculate the antilog_{10} of the results (see **8.2.8** and Table E.3).

Table E.3 — Results in minutes

	Reference dispenser	Candidate
Mean	6.0	7.3
Ratio of means	1.22	
95 % confidence limits of ratio	(0.94, 1.58)	

E.3 24 h knockdown results

E.3.1 An example of the calculation of 24 h knockdown results, to be carried out on a step by step basis, is given in **E.3.1** to **E.3.7**, using the values in Tables E.4 and E.5.

Table E.4 — Example of 24 h knockdown results

Percentage		Arcsin scale	
Reference dispenser	Candidate	Reference dispenser	Candidate
95	75	77.1	60.0
97	93	80.0	74.7
85	85	67.2	67.2
98	80	81.9	63.4
90	90	71.6	71.6
93	70	74.7	56.8

E.3.2 Calculate the mean arcsin results and standard errors (see **8.3.3**), as shown in Table E.5.

Table E.5 — Mean arcsin results and standard errors

	Reference dispenser	Candidate
$\bar{x}$	75.4	65.6
se($\bar{x}$)	2.22	2.80

E.3.3 Calculate the difference, δ, between the reference and the candidate dispenser (see **8.3.4**).

$$\delta = 75.4 - 65.6$$
$$= 9.8$$

E.3.4 Check the homogeneity of variances (see **8.3.5**).

$$\frac{2.8^2}{2.22^2} = 1.59$$

1.59 is less than 7.15, therefore continue the analysis.

E.3.5 Calculate the standard error of the difference, se(δ), (see **8.3.6**).

$$\text{se}(\delta) = \sqrt{(2.22^2 + 2.80^2)}$$
$$= 3.57$$

E.3.6 Calculate the lower and upper 95 % confidence limits of the difference, L_{95} and U_{95} (see **8.3.7**).

$$L_{95} = 9.8 - (2.228 \times 3.57)$$
$$= 1.8$$
$$U_{95} = 9.8 + (2.228 \times 3.57)$$
$$= 17.8$$

E.3.7 Backtransform the mean results and calculate the difference in percentage (see Table **E.6**).

Table E.6 — Results in percentage

	Reference dispenser	Candidate
Mean	93.6	82.9
Difference	10.7	

E.4 Interpretation of results

On average, the KT_{50} value for the candidate is greater than that of the reference dispenser by a factor of 1.22. However, the data also support the assertion that the KT_{50} value of the candidate could be as much as a factor of 1.58 greater. On average, the percentage 24 h knockdown value of the candidate is 10.7 % less than that of the reference dispenser.

Given the assessment criteria described in **8.4**, as both the U_{95} values are greater than their respective target values (A = 0.176 and B = 10° as specified in BS 4172-1:1998), the insecticidal performance of the candidate is rated as "worse than" the reference dispenser and therefore does not conform to the respective performance requirements. However, as both of the L_{95} values fall below their respective target values, it may be worthwhile including more dispensers, repeating the calculations using all determinations (see **E.5**).

E.5 Illustrations of the conformity criteria

An example of conformity conditions, with candidate results that are just sufficient to conform to chosen values for A and B, is given in Tables E.7 and E.8. Illustrations of the three broad categories of possible results are shown in Figures E.1 to E.3.

For the performance of the candidate to be rated as "not worse" than that of the reference (see BS 4172-1, clause **4**) the U_{95} values for both variables have to fall to the left of their respective target values for A and B, i.e. 0.176 or 10°.

E.6 Critical values

Critical values are chosen from Tables 1 and 2 (see **8.2**) and based on the number, n, of dispensers used for the reference dispenser or candidate. In the example in this annex there were six dispensers so the respective critical values for the distribution F and the Student's multiplier t were 7.15 and 2.228.

Table E.7 — Examples of KT_{50} results just sufficient to achieve conformity

Conformity criterion for U_{95} on $\log_{10}$ scale	Example	Mean result of reference dispenser min	Maximum value of mean of candidate[b] min	Results for U_{95}
$U_{95} \leq 0.176$[a]	i)	4	5.2	0.170
	ii)	6	7.9	0.175
NOTE The formula estimating the U_{95} value upon which the conformity is based shows how the variability of results has been taken into account (see Foreword) i.e. the greater the variability, se(δ), the lower the candidate mean has to be in order to conform. It also shows the benefit of increasing the number of dispensers in the reference and candidate samples; as numbers increase, t decreases allowing $\bar{x}_c$ to increase while maintaining conformity.				
[a] Where $U_{95} = (\bar{x}_c - \bar{x}_r) + t \times \text{se}(\delta)$ (see **8.2**, **8.2.7**). [b] Assuming six replicates of both reference dispenser and candidate and se(δ) = 0.025 for $\log_{10}$ KT_{50}.				

Table E.8 — Examples of 24 h knockdown results just sufficient to achieve conformity

Conformity criterion for U_{95} on arcsin scale	Example	Mean result of reference dispenser %	Maximum value of mean of candidate[b] %	Results for U_{95}
$U_{95} \leq 10°$ [a]	i)	50	42.3	9.999
	ii)	97	93.9	9.895
NOTE The formula estimating the U_{95} value upon which the conformity is based shows how the variability of results has been taken into account (see Foreword) i.e. the greater the variability se(δ) the higher the candidate mean has to be in order to conform. It also shows the benefit of increasing the number of dispensers in the reference and candidate samples; as numbers increase, t decreases allowing $\bar{x}_c$ to increase and still conform.				
[a] Where $U_{95} = (\bar{x}_r - \bar{x}_c) + t \times \text{se}(\delta)$ (see **8.3**, **8.2.7**). [b] Assuming six replicates of both reference dispenser and candidate and se(δ) = 2.5 for arcsin 24 h knockdown.				

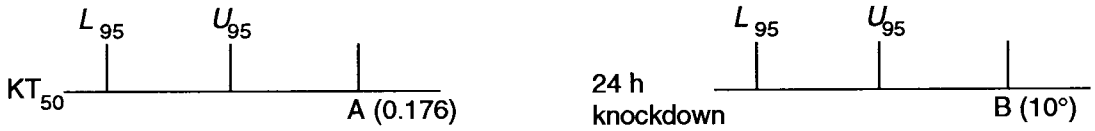
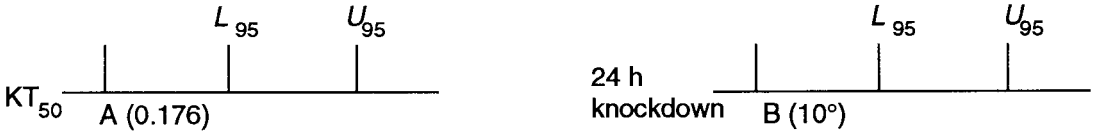


Figure E.1 — Candidate passes the assessment



NOTE If L_{95} is greater than either 0.176 or 10° then there would be very little likelihood of improving the overall test result by testing more candidates, in this case the candidate has effectively failed the assessment.

Figure E.2 — Candidate fails the assessment

NOTE If both values of L_{95} are less than 0.176 or 10° the use of more dispensers may result in conformity.

Figure E.3 — Indeterminate outcome demonstrating need for an extended study

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Standards publications

BS EN 60825, *Safety of laser products*.

Other publications

[1] EUROPEAN COMMUNITIES 80/232 EEC. Prescribed Quantities Directive 1980.

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